Lab-on-a-chip: microfluidics in drug discovery

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Abstract | Miniaturization can expand the capability of existing bioassays, separation technologies and chemical synthesis techniques. Although a reduction in size to the micrometre scale will usually not change the nature of molecular reactions, laws of scale for surface per volume, molecular diffusion and heat transport enable dramatic increases in throughput. Besides the many microwell-plate- or bead-based methods, microfluidic chips have been widely used to provide small volumes and fluid connections and could eventually outperform conventionally used robotic fluid handling. Moreover, completely novel applications without a macroscopic equivalent have recently been developed. This article reviews current and future applications of microfluidics and highlights the potential of 'lab-on-a-chip' technology for drug discovery.

Electrophoresis
The motion of charged particles in an electrical field towards the opposite electrode.

Photolithography
A fabrication technique to generate small features in micrometre dimensions on microchip substrates such as silicon, glass or polymers.

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The search for drugs demands robust and fast methods to find, refine and test a probable drug candidate. The discovery of the elusive molecule with unique qualities out of a nearly unlimited number of possibilities is laborious, time consuming and relies heavily on technological resources that are available for handling small liquid volumes, automation, and high-throughput processing and analysis. An important advance was the miniaturization of entire systems, that is, the introduction of high-density plate formats and nanolitre dispensing systems. The required procedures, however, are extremely diverse and include manifold syntheses and analytical steps *in vivo* and *in vitro*. Performing these processes on a microfluidic chip in a continuous stepwise manner could pave the way for increased speed and automation opportunities.

Microfluidic chips are small platforms comprising channel systems connected to liquid reservoirs by, for example, tubing systems in turn linked to syringes. The size of the channels is in the range of a few micrometres, which greatly facilitates handling of volumes much smaller than a microlitre. Appropriate channel design and integrated tools such as electrodes or a specific surface pattern are now facilitating the incorporation of many operational steps by allowing molecular compounds to pass each unit successively (BOX 1). These individual steps include sampling, sample enrichment (pre-concentration and preconditioning steps, such as filtering), mixing, reaction modules (for example, different heating zones), product separation, isolation and analysis.

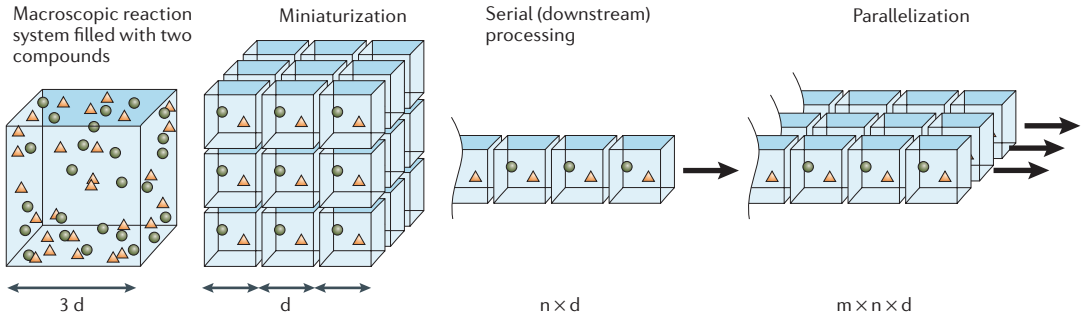
Initially, the concept of microfluidics was dedicated to significantly reducing sample consumption and increasing efficiency in separation methods[1]. A prominent example is the miniaturization of electrophoresis and related techniques[2–4]. The high efficiency of electrophoretic separation in channels with small cross-sectional area is achieved through improved heat transfer, and the simplicity of the planar design allows for massive parallelization[5]. Furthermore, the low costs of mass producing microchips and the automation of reaction systems have allowed the adaptation of microfluidic systems for commercial use. Custom-made planar microfluidic chips and entire measuring systems are now commercially sold by companies such as Agilent Technologies, Evotec Technologies, Caliper Life Sciences, Hitachi and Fluidigm Technology[6].

The fabrication of microfluidic chips usually requires cleanroom facilities and specific equipment (see REF. 7; see MEMS and Nanotechnology Clearinghouse, Further information). A great variety of microchip fabrication techniques and materials are available for producing highly sophisticated two- and three-dimensional microstructures with integrated modules. Pumps and valves, mixers, motors and other functional units that assist chemists in the macroscopic lab have been miniaturized[8,9]. Likewise, sensors and detectors — even optical components — can be integrated on-chip[10]. The vast majority of microfluidic devices are, however, simple planar microchips fabricated by photolithography on substrates such as glass, silicon or polymers[11–14].

Box 1 | **What is gained by miniaturization?**

Some simple considerations show what various opportunities can emerge from miniaturization of reaction systems. This is illustrated by means of scaling laws (figure and table) giving the dependence of reaction parameters on the size of the system (for example, reaction volume and diffusion time)[1,25]. Due to the short distances in microfluidic channels, the transport times of mass and heat are shortened. Fast and controlled heat supply as well as cooling is facilitated due to high surface to volume ratio. Therefore, important running conditions of chemical processes, such as compound concentration and temperature, can be regulated precisely. One key feature of microfluidics is the integration of different functional units for reaction (for example, mixer and heater), separation and detection in a channel network. Therefore, serial processing and analysis can be easily performed in the flowing systems. Furthermore, because space is used sparingly, massive parallelization can be accomplished.

d, length of edge; n and m, numbers of reaction systems serial and parallel, respectively.



Parameter	Macroscopic example	Factor change	Microscopic example
Length of edge	1 mm	d	1 µm
Surface	1 mm^2	d^2	1 $µm^2$
Volume	1 µl	d^3	1 fl
Number of molecules	10^9	d^3	1
Diffusion time over d (D = 10^{-6} cm^2s^{-1})	15 min	d^2	1 ms
Example: in flowing systems			
Linear flow rate	1 µm/s	d	1 mm/s
Separation time	10^5 s (>1 day)*	d^2	100 ms
Example: in planar array			
Number of volumes per microwell plate	96	d^2	10^8

*Typically, for example, high-performance liquid chromatography in packed column.

Tightly linked to miniaturization technology is the capacity to image and detect the sample. Reduction of the reaction volume naturally decreases the absolute number of analytes and therefore makes possible the identification and analysis of individual small compounds out of a flowing bulk sample. Therefore, adequate, high-sensitivity detection techniques are indispensable.

Microfluidic systems have been adapted and interfaced to most of the common analytical detection techniques, such as electrochemical methods, mass spectrometry and optical methods including absorption, refractive index variation, surface plasmon resonance, chemoluminescence and fluorescence.

By far the most popular approach for high-sensitivity detection is fluorescence analysis. The high temporal resolution enables real-time measurements. Moreover, fluorophores attached to the analytes can be excited and detected selectively. The excellent sensitivity of fluorescence spectroscopy is further enhanced by reducing the size of the detection volume, which results in high signal-to-background ratios, such as that achieved by confocal setups (laser scanning microscopes) and total internal reflection microscopy. Analysis on the single-molecule-level in microchips is possible for various applications[15] such as enzymatic assays[16], kinetic studies of protein folding[17] and photo-induced protein conversion[18] and, in particular, for the detection and analysis of DNA[19–23].

'Lab-on-a-chip' technology is an emerging field, approached by scientists from many disciplines (BOX 2) and exploited for a wide range of applications. The myriad benefits of microfluidic platforms for the study of biological systems, as well as details of fabrication procedures for generating microfluidic chips, have been reviewed in a number of excellent articles[8–15,24–29].

The following review highlights current and possible future applications of microfluidics in the process of drug discovery, and focuses on the developments for chemical syntheses, high-throughput screening and cell treatment. FIGURE 1 summarizes the various process steps that can be performed by microfluidic methods, and some of their applications to drug discovery.

Total internal reflection microscopy
An optical method to image fluorescent samples at interfaces, such as in proximity to a glass surface.

Chemical syntheses for compound generation

Although the majority of studies make use of the analytical potential of the microfluidic chips, considerable efforts have also been made to use microsystem technology

Box 2 | **Definitions related to 'lab-on-a-chip' technology**

'Lab-on-a-chip' technology in the life sciences is a research area that is approached by scientists from different disciplines each having its own 'language' and standards. In the course of time, however, several buzzwords have emerged that are nowadays used in parallel. In the following, we give definitions for the most often mentioned words, being aware that the meanings overlap.

μTAS (micro total analysis system)
An integrated system that performs all steps in an analysis — that is, sampling, sample pre-treatment, sample transport, chemical reactions, analyte separation, product isolation and detection in a microfluidic chip. First defined in 1990, μTAS emphasizes the analytical function of a microfluidic chip.

'Lab-on-a-chip'
Lap-on-a-chip is a term widely used for any kind of research with the goal of miniaturizing chemical and biological processes. It is not a well-defined scientific term. Lab-on-a-chip technologies include microfluidic chips as well as non-fluidic miniaturized systems such as sensors and arrays (the so-called biochips).

Miniaturization
The fact that existing molecular assays, such as chemical binding, chromatographic extractions and electrophoretic separations, can be performed in different volumes without a difference in chemical quality (identical information content) has led to a variety of technologies of fabrications that enable reactions to be scaled-down or 'miniaturized'. Miniaturization is most commonly aimed at particles (beads, packed columns and vesicles), capillaries, gels and fluidic chips.

Microfabrication
Microfabrication describes 'cleanroom' fabrication processes for engineering solid flat substrates. It includes photolithography, direct-write laser (e-beam, X-ray) lithography, wet etching, reactive ion etching, film deposition (plasma, chemical vapour, sputtering) and bonding (eutectic, anodic, thermal, adhesion). It is mostly used for micro-electronic circuit fabrication, but also for MST or MEMS (see below) and 'lab-on-a-chip'.

Microsystem technology (MST) and micro-electromechanical systems (MEMS)
The word MEMS is taken from the electronics industry. MEMS refers to the fabrication of electronic or mechanical components on chips. Similarly, bio-MEMS emphasizes the integration of functional units (particularly electrical manipulation processes) on microchips for biological and biomedical purposes. The term also includes biomedical devices such as chips for minimally invasive surgery and microneedles. In Europe, MST is used to describe MEMS.

Microfluidics
Microfluidics signifies any kind of experimental and theoretical research of liquid streams generated in chips comprising microsized channels, including fabrication, handling and practical use of these chips. In a broader sense, streams of gases or fluidized solids/particles in microchips are also included in the definition of microfluidics. For channels with dimensions in the sub-micrometre range, the word 'nanofluidics' is used.

(MST; also known as MEMS, see BOX 2) for preparative applications. In the following section advances in synthetic processes, in particular the production of combinatorial libraries, are described.

Using merging channel geometries (such as T and Y shapes), various reactants can be mixed and intermolecular reactions induced. The concentrations of reagents can be regulated in space and time, which gives rise to an additional level of control. Moreover, temperature — a key parameter in chemical processes — can be accurately regulated. The improved heat and mass transfer in small-scale reactors justifies positive expectations for preparative applications in microreactors. Indeed, many syntheses have been successfully performed in microreactors. Numerous examples demonstrate the benefits of miniaturizing reactors such as the significantly shortened reaction time — for example, from hours to minutes[30,31]. In addition, many syntheses in microreactors yield higher conversion rates and improved selectivity[32]. A generalization of the results is, however, not admissible and must be drawn for every chemical reaction separately.

In continuous flow microreactors, multi-step reactions can be performed easily. One promising example by Watts *et al.* is the multi-step synthesis of dipeptides giving a substantive yield over a period of 20 minutes[33] instead of a 24-hour procedure in a batch reaction yielding 40–50%. In a follow-up study the group demonstrated the formation of tripeptides[34]. They also found reduced racemization in a microreactor synthesis compared to a batch reactor[35].

Operating under continuous-flow conditions also allows the combination of multiple reaction steps and on-line analysis on a single chip. Shortened reaction times and the direct analytic functioning of a chip could result in faster optimization of reaction parameters (feedback control). Furthermore, this concept of micro synthesis and total analysis system (μSYNTAS) provides a highly effective route for solution-based combinatorial chemistry first demonstrated by Mitchell *et al.*[36]. In this work, a sub-reaction of the Ugi multicomponent condensation was performed in a distributive micromixing device that was integrated with time-of-flight mass spectrometry. The reaction was carried out between five different secondary amine hydrochlorides (piperidine derivatives) and formaldehyde to produce five different iminium cations. The injection of the piperidine derivatives into a methanol solution of formaldehyde was performed in a sequential manner (serial mode), as well as simultaneously (parallel mode) in a separate procedure. For both modes, the compound library could be derived and fully analysed in real time. The formation of a larger 7 × 3 combinatorial library was also demonstrated. For formation of 21 pyrazole derivatives (Knorr synthesis), seven different dicarbonyl reactants and three different hydrozines were supplied sequentially into a glass microreactor[37]. No evidence of cross-contamination or carry-over was detected between the two consecutive reactions.

Solution-phase combinatorial synthesis of (n × m) products (from two libraries with the size of n and m compounds, respectively) in a parallel fashion requires the respective number (n × m) of separated reactors (FIG. 2). As a consequence, the complexity of such systems grows rapidly with the number of compounds[38–40]. Using microchip technology, the need for large space and large quantities of sample can be eliminated, which was shown for a 2 × 2 reaction scheme by Kikutani *et al.*[39]. They performed combinatorial formation of amides from two different amines and two different acid chlorides. The main challenge of the work, however, was the fabrication of a microfluidic chip that facilitates all possible mixing combinations for any n × m system (while the number of starting compound reservoirs are n + m). It is topologically impossible to disentangle all mixing modules in a system with more than two compounds on a planar chip, which necessitated the fabrication of a three-dimensional channel network.

Ugi multicomponent condensation
An organic reaction between a ketone or aldehyde, an isocyanide, an amine and a carboxylic acid to form a bis-amide. Libraries of low-molecular-mass drug-like compounds can be generated via the Ugi multicomponent condensation.

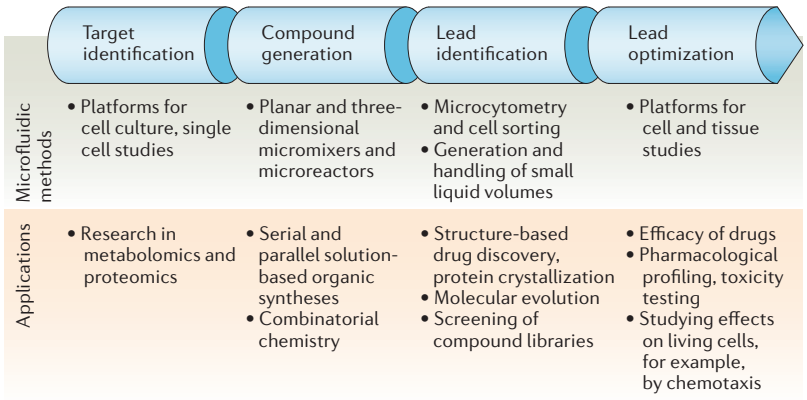


Figure 1 | **Microfluidics in drug discovery.** The figure depicts microfluidic methods, including respective applications, that are valuable for individual steps in the drug discovery process.

Despite the various examples, the potential of syntheses in microchips has not yet been fully realized. A novel approach demonstrating the potential of microfluidic channels was provided recently[41], in which dry powder with large particle size (>100 μm) was injected into a microchip. By gaseous sheath flow, the powder was fluidized and could be transported through the microchannels. Such devices with reliable particle feeding systems could potentially replace the time-consuming process of weighing powders using a balance.

High-throughput screening and cell sorting

Having produced a library of compounds, the next straightforward step is lead identification. The search for compounds that bind to a particular target molecule, or interfere with, catalyse or modulate a particular reaction, could be performed *in vitro* or *in vivo*. In any case, methods and tools are required that are capable of vetting an extremely large number of compounds in a short time[42,43], because the number of possible leads is so vast.

The main efforts to save time and chemical compounds in high-throughput screening applications have resulted in miniaturization of existing technologies — that is, in increasing the well density on microwell plates, thereby decreasing the size of each well. However, currently available robotic systems are limited with regard to the speed of fluid dispensing that is achievable, and the accurate handling of very small liquid volumes that evaporate quickly is another challenge to this approach[44,45]. Alternatively, screening assays can be performed in a continuous manner using a flowing system that allows the analysis and isolation of compounds on a single chip[46]. The microfluidic channels form a sealed reaction system. After the introduction of compounds, liquid handling is performed exclusively within the channel system, which renders pipetting procedures unnecessary.

Examples for miniaturized continuous-flow separation devices are often based on physical properties of the analytes, such as electrophoretic mobility[47], refractive index[48], density[49] or size[50,51]. On the other hand, dissolved or suspended particles and cells can be manipulated within the solution, and uncoupled from their molecular and cellular properties, which makes them amenable to various kinds of secondary control parameters. For example, utilizing fluorescence is widely applicable for screening of libraries (such as phage-display technology[52,53]) and for viability tests (for example, determination of toxicity), as well as for clinical medicine and basic biological research.

Much effort has been made to develop microsized fluorescence-activated cell-sorting devices (μFACS)[54–63] (microfluidic chips for flow cytometry are also commercialy available; for example, Agilent 2100 Bioanalyzer or Cytocon (Evotec Technologies)). The main challenge in such devices is the integration of sorting modules for precisely controlling the direction of the cells when a target cell is detected. One of the first examples of a chip-based sorting device was introduced by Fiedler *et al.* in 1998 using dielectrophoresis (DEP)[54]. Certain electrode configurations were constructed to function either as a funnel, as aligners to break aggregates of cells, as a caging module for cell trapping or as a switching module. Recently, Hu *et al.*[55] adapted the DEP principle and presented a cell-sorting device that is capable of selecting rare bacterial cells that are attached to a DEP-responsive label.

In 1999, Quake and co-workers developed a microfluidic chip capable of detecting and sorting stained DNA fragments[56] and fluorescent bacterial cells[57] with high-throughput rates. The sample was guided by electro-osmotic flow at a T-junction into one of the output channels by proper switching of the electrodes. In following work, a further cell-sorting device was presented in which the transport of cells and sorting modules were solely based on hydrodynamic pumps[58]. Furthermore, a sorting module based on optical forces was reported. In this work, a Y-shaped channel was utilized. Flow to the output channel was asymetrically biased such that all cells flowed to the waste channel. When a target cell was identified, the optical switch was activated and a focused laser spot deflected the cell to the target output channel[59].

Although the throughput rates of μFACS devices (maximum several hundred cells per second) are still below commercial FACS machines (with impressive speed of several 10,000 cells per second), they offer the benefits of a continuous operation, including reaction steps preceding and after the sorting process. One example demonstrating this was introduced in 2003[60]. The sorting parameter was the affinity of a fluorescent protein (phycoerythrin) for the bacterial cell membrane performed on the chip prior to the detection of the cells. This chip was adapted to a confocal microscope and was thereby capable of ultra-sensitive detection on a single-molecule scale.

The combination of appropriate biological assays and high-sensitivity detection techniques with such systems will allow identification and isolation of individual cells or molecules[64]. Moreover, the analysis of interactions among molecules that have already been identified as diagnostic or therapeutic targets is facilitated. By attaching target molecules to microspheres (beads), the selection of binding molecules from libraries to peptides/proteins, for example, could be performed[65].

Dielectrophoresis
The repulsion or attraction of particles in a non-uniform electrical field based on polarization effects.

Electro-osmotic flow
A method to induce flow in a microchannel. An ionic double layer is present at the interface of the (immobile) microchannel (with charged surface) and the mobile fluid (counter-ions are accumulated near the channel surface). Application of an electrical field along the microchannel causes the dissolved ions, together with the bulk fluid, to move to the respective electrode.

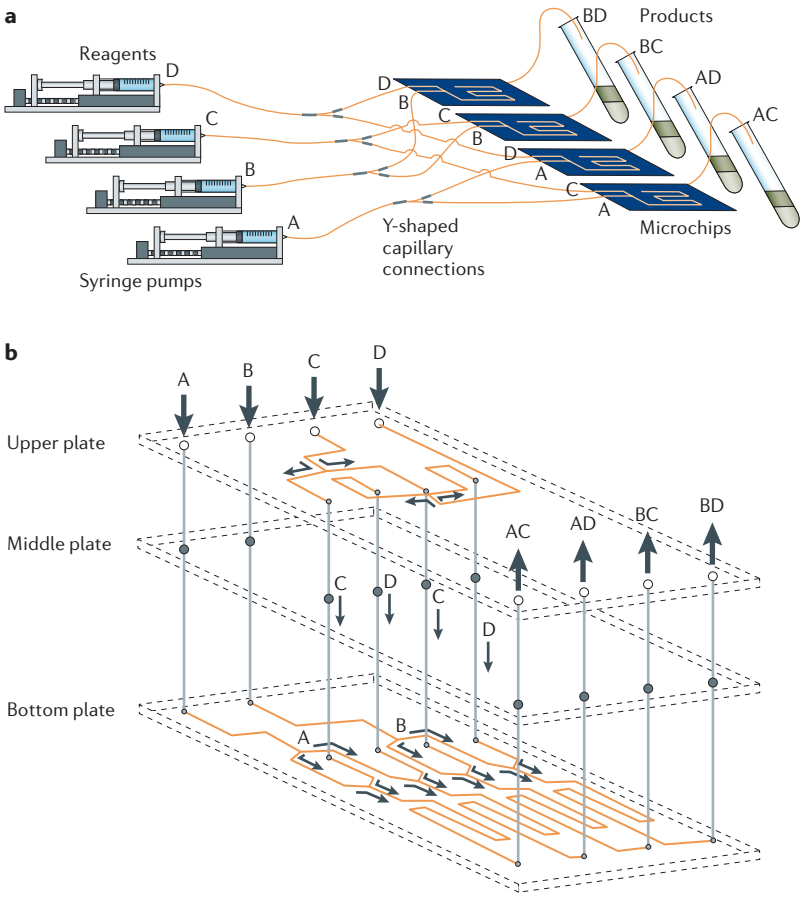


Figure 2 | **Combinatorial syntheses in microfluidic chips. a** | Schematic views of a microreactor system for 2 × 2 combinatorial syntheses composed of four microchips. **b** | Schematic views of a microreactor system for 2 × 2 combinatorial syntheses realized in one single three-dimensional microchip[39].

Microfluidic generation of small liquid volumes

Screening for products of any intermolecular reaction between probable leads and targets can be facilitated in reaction mixtures that are enclosed in small containers such as liposomes[66] or emulsion droplets. In microfluidic chips, small (nano- and picolitre) liquid volumes can be generated by the formation of aqueous droplets in a carrier medium, such as a hydrophobic compound or a gas. The ability to precisely control the supply of reagents, to handle small liquid volumes without fast evaporation, and the high-speed formation of droplets with homogeneous diameters of a few μm makes this a valuable tool for screening experiments that rely on high reproducibility such as protein crystallization[67] and molecular evolution[68].

By injecting the aqueous phase into the stream of the carrier medium at a T-junction[69] or by applying focusing techniques[70] (FIG. 3a,b), small entities with a volume of femtolitre to nanolitre are generated with an exceptional reproducible size that is far beyond the capability of former techniques for emulsification. The droplets form small reaction containers that can be filled with (various) compounds at the very moment of formation. Unlike miniaturized continuous-flow reactors, the generated droplets form a boundary in three dimensions. The tiny, finite volume enables rapid mixing of reactants and transport along channel networks[71].

Since its first demonstration in microfluidic chips in 2001[69], the generation of segmented flow has attracted broad interest. Segmented flow has been used among other techniques to carry out chemical and enzymatic reactions, including PCR[72] and kinetic studies[73,74], and in the formation of small particles[75,76]. It was also used for cell analysis by encapsulating a single cell within one microdroplet in which the cell was later lysed and analysed[77].

Plug-based systems can probably be adapted for structure-based drug discovery, in which protein crystallization has remained one of the rate-limiting steps in determining three-dimensional macromolecular structures[78,79]. The small volumes result in a significant reduction in the amount of starting material required, and the environmental conditions can be adjusted very accurately. Ismagilov and co-workers[80] used aqueous droplets with a volume of ~10 nl generated on a microfluidic chip to determine optimum crystallization conditions. Evaluation of the quality of the crystals was subsequently performed by X-ray diffraction[81]. In following work, an array of nanolitre plugs of many different reagents was produced that, in a second step, merged with an aqueous stream containing another compound. The usefulness of this approach was demonstrated by functional assays in which a set of enzymes was screened for phosphatase activity. Furthermore, screening of a protein (thaumatin) against multiple crystallizing agents was shown[82]. Preformed plugs were filled with 48 precipitants and merged with a stream of thaumatin, thereby giving rise to 48 crystallization trials.

Aqueous droplets are also a useful tool for molecular evolution[68], a method that is particularly interesting for the optimization of drug candidates. Aqueous droplets form cell-sized compartments that keep together the genes, the RNAs and proteins that they encode, and the products of their activities. *In vitro* expression of green fluorescent protein (GFP) to directly analyse the contents of individual droplets has been achieved on a microfluidic chip[83] (FIG. 3).

Having generated the droplets, different methods of droplet handling and manipulation such as fission, fusion and sorting[84,85] could be performed. For example, in a sorting module as sketched in FIG. 3a specific droplets can be isolated from the main droplet array.

For all the above applications, a simple planar microfluidic chip is sufficient; the addition of tubings, fittings, syringes and pumps makes the device complete. The simplicity of this set-up is perhaps the reason why there has been extremely fast adaptation of the technology for further applications. Future developments, such as enhancement of droplet formation and integration of different modules, can undoubtedly be expected in the short term.

Continuous droplet formation is one way to create small volumes; the use of micromechanical valves is another. Valves can be independently manipulated and in this respect are advantageous to the previously described droplet-based systems. Appropriate fabrication

Focusing techniques
Narrowing of a fluid stream by applying a sheath flow, for example, in a crossed channel geometry.

Segmented flow
Flow of alternating plugs (droplets) of two immiscible liquids or a liquid and a gas.

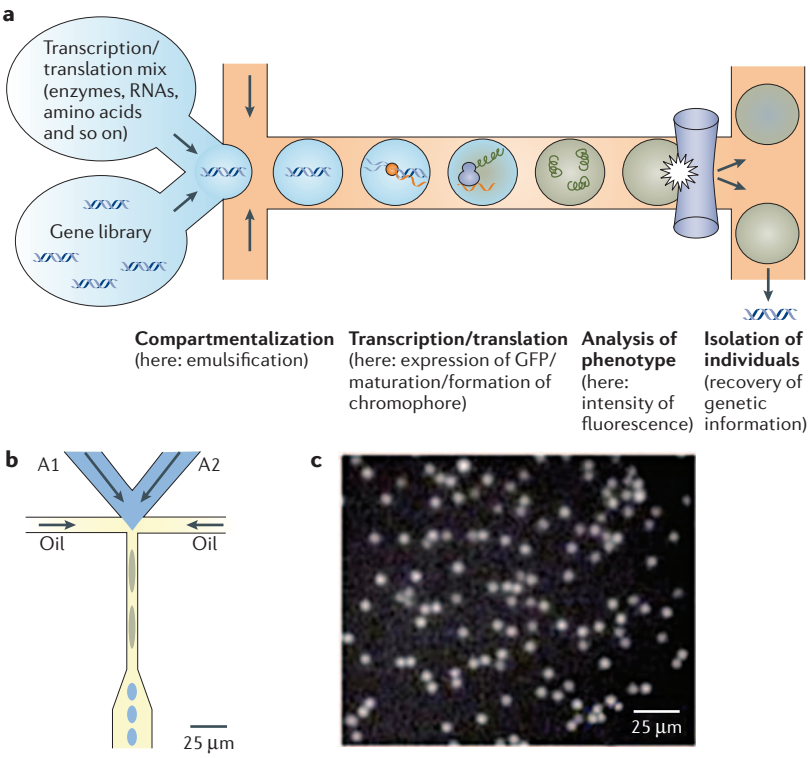


Figure 3 | **Screening in microdroplets. a** | The scheme of the concept of *in vitro* evolution of proteins in a microfluidic channel. **b** | A scheme of the microchip (A1 and A2: aqueous solution containing the translation/transcription mix and a green fluorescent protein (GFP)-encoding plasmid, respectively). During continuous formation of a water-in-oil emulsion, the molecules required for cell-free expression of proteins are mixed with templates from a gene library. *In vitro* expression takes place within the droplets. Analysis could be directly performed and distinct droplets could be isolated by appropriate sorting units within the microfluidic channel. **c** | In the experiments, GFP was used as a model system. After the autocatalytic formation of the chromophoric group, GFP inside the droplets could be determined by fluorescence spectroscopy. Part **c** reproduced, with permission, from REF. 83 © Wiley (2005).

processes, however, are necessary to integrate mechanical valves into the microchannel. The construction of a simple valve is realized by multilayer soft lithography[86]. The chip consists of two layers comprising microsized channels. The bottom layer contains the microfluidic channel network, and the other one, situated on top, represents the control layer. By applying pressurized gas in the channels of the control layers, the bottom layer is closed at the intersection of top and bottom channels. Highly complex valve patterns could be constructed to be used, for example, for fluid metering in protein crystallization studies[87] and high-throughput screening of fluorescence-based single-cell assays[88]. Microchips comprising such mechanical valves have been developed for the commercial market and are currently sold by Fluidigm Technology.

Microfluidic cell treatment

The testing of compounds on living cells is an important part of the drug discovery process, but optimal drug testing requires conditions that are as close to the physiological context as possible. In microfluidics, the microscale dimensions generated are approaching reaction volumes that are typically found in biological systems. Microfluidic devices therefore make it possible to manipulate single objects of cellular size, and so analysis under controlled yet physiologically relevant environments can be achieved. Moreover, by parallelization of applied methods large numbers of cells can be observed simultaneously —that is, under comparable conditions.

Using microfluidics, cells can be stimulated with spatiotemporal resolution to study, for example, the effect of drug levels on chemotaxis of living cells *in vitro*. It is even possible to deliver reagents to defined domains of the cell surface or cell interior rather than to expose them to the whole cell. Such studies can reveal cell dynamics and phenomena based on (sub)cellular heterogeneity. One simple approach to partial treatment of cells was demonstrated by Takayama *et al.*[89], in which mammalian cells were allowed to settle onto the floor of a microfluidic channel. Localized perturbation of the cells was achieved by creating parallel streams of different solutions flowing across the cells. Due to a laminar flow, the solutions only mix slowly by diffusion. By this procedure, labelling of subpopulations of mitochondria, as well as disruption of actin filaments in selected regions of the cells, was demonstrated. In such devices, the number of parallel streams that can be generated is, in principle, unlimited. Due to diffusion, however, the boundaries of narrow parallel layers are blurred shortly after generation. On the other hand, diffusion at the boundary allows the formation of gradients. In appropriately designed channel networks gradients with linear, parabolic or periodic shape were generated[90]. The concentration differences could expand over several orders of magnitude and over several different compounds. Applied to cell culture experiments, it allows for rapid acquisition of data for cellular responses depending on the concentration of the compounds the cells are exposed to[91].

The exploitation of multiple laminar streams is not limited to gradients of chemical compounds. Recently, the generation of a temperature step of up to 10 °C was demonstrated by converging two aqueous streams, one of which was heated separately while the other was cooled[92]. In this work, *Drosophila* embryos were positioned at the interface of the two temperature-regulated streams so that the anterior and posterior halves were exposed to different temperatures and therefore developed at different rates.

The formation of networks by laminar streams is an attractive approach for local stimulation of cells, because microfluidic devices comprising a simple two-dimensional network of channels can be fabricated at reasonable cost. The perturbation of the cell happens, however, along the length of the cells, which is to say that the resolution is not three-dimensional.

Another interesting approach for the stimulation of single cells was illustrated by Peterman *et al.*[93], using a microchip with small apertures connected to separate microfluidic channels. Driven by electro-osmotic flow, minute quantities of a chemical compound (bradykinin solution) were repeatedly delivered to PC12 cells (a neuronal cell line) adhering to a chip surface, therefore mimicking a biological synapse. The apertures with a

Laminar flow
In the laminar flow regime, no turbulence is observed. As a consequence, two merging fluid streams are flowing in parallel, so that mixing occurs only by diffusion.

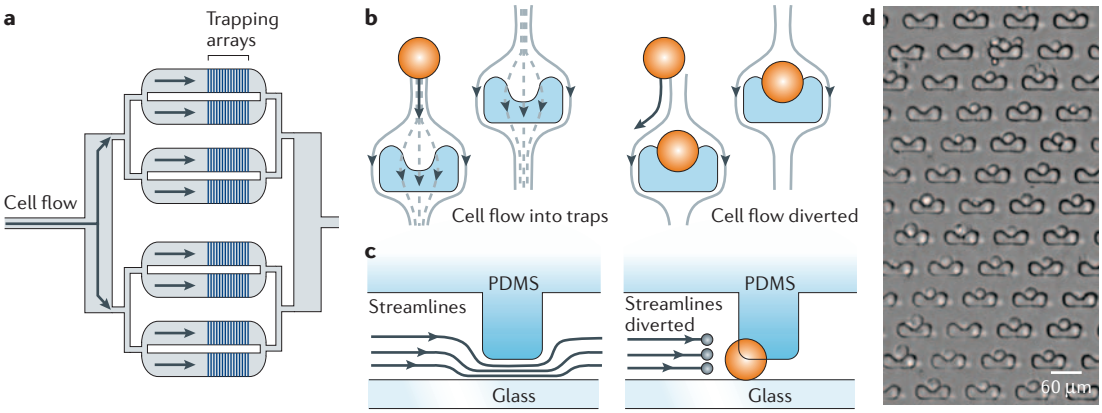


Figure 4 | **Trapping cells for large-scale single-cell analysis. a** | Sketch of the chip. A cell suspension is introduced to the chip and guided to various trapping arrays. Due to the appropriate size of the traps, only one cell can occupy the space, while succeeding cell pass by. The feature, fabricated in PDMS, is designed in such a way that only the cell would be trapped while the buffers continously flow. Parts **b** and **c** show schemes of the trapping regions, top and side view, respectively. **d** | Trapping of HeLa cells. (Courtesy of L.P. Lee, University of California, Berkeley).

diameter of 5 µm allowed the cells to be investigated on a single-cell level. Decreasing the size of the aperture could further improve the spatial resolution.

Positioning of cells is usually random, and they adhere at any place in the microfluidic channel simply by settling down. Controlled positioning of cells can be facilitated by a pattern on the surface of the channel, resulting in spots of preferential adhesion on the channel's floor. In a novel approach towards array cytometry, cells were held by obstacles incorporated into the channel[94] while the fluid passed above and at both sides of the cells in the microfluidic channel (FIG. 4). Using obstacles of appropriate shapes and sizes, just one cell per obstacle was trapped, which was applied for studies of enzyme kinetics on a single-cell level.

A growing number of studies are using microfluidic platforms to observe long-term effects and slow cell responses. For this, the microfluidic chip has to be adapted to the needs of living cells. Challenges for cell culturing include the creation and maintenance of required conditions, such as constant temperature and nutrient concentration, and the prevention of metabolite accumulation. Very recently, several approaches have been presented illustrating cell culture experiments of different cell types — bacterial and yeast cells[95,96], endothelial[97], nerve[98] and muscle cells[99] — enabling long-term observation of cell growth, differentiation and response. Moreover, there have been promising advances in the design of programmable pumps and valves that could be used to precisely control liquid supply in cell culture experiments[100].

The possible increase of complexity of such devices, which have the potential to replace animal testing, was demonstrated by Shuler and co-workers[101]. A system designed to mimic cell culture was fabricated on a microchip to allow studies of the effects of chemical exposure on cell metabolism. Mammalian cells were cultured in different compartments of a microchip to represent organs ('lung', 'liver' and 'other'), which were in turn interconnected by microfluidic channels acting as blood surrogate. A sensor for detecting dissolved oxygen was furthermore integrated into the chip.

The above mentioned cell culture applications of microchips are certainly still at the development stage. But knowledge gained from these examples will help to construct reliable and maybe fully portable cell culture platforms that include automatic regulation of environmental conditions.

Conclusions

The adaptation of microfabrication technology to analytical sciences has provided much scope for new ideas and experimental applications during the past decade. The large body of literature detailing applications of microfluidics beyond proof-of-principle studies demonstrates that the technology is maturing from its infancy. Some challenges still remain, however, before microfluidics platforms can be used to adapt or replace existing assays. Standardization of materials, interfaces and channel geometries will improve the comparability and assessment of experimental procedures and results. Moreover, ease of handling and robustness of systems have to be refined — 'as simple as possible' is certainly a valuable motto in the field of microfluidics.

The development and success of drug discovery is crucially dependent on available technologies. In key areas of drug discovery, such as chemical syntheses, screening of compounds and preclinical testing of drugs in living cells, microfluidic tools can make a useful contribution, and indeed represent an improvement on existing technologies. As discussed, novel reaction, manipulation and analytical steps can be performed with microfluidic systems that are not accessible to other approaches because the key tools for manipulation inevitably have to be of similar dimensions to the analysed sample. Therefore, microfluidics offers innovative technological opportunities for obtaining new information about biological systems.

The power of these methods can only be fully realized if biological assays are developed and adapted effectively to the microscale. A synergistic effort both

Interfaces
The connection between a microchip and its features to a macroscopic system, such as microscopic stages, tubing systems and wiring interconnections.

by developers of conventional biological assays and those working on miniaturization will surely aid the adoption of microfluidics technology by academic and industrial life scientists in the near future. If this succeeds we can expect exciting technological breakthroughs in the near future.

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